II. Biochip Cartridges

Bottom Substrate

[0123] The biochip cartridges of the present invention include a solid substrate containing a number of functionalities for use in the present invention. By "substrate" or "solid support" or other grammatical equivalents herein is meant any material that can be modified to contain discrete individual sites appropriate of the attachment or association of capture ligands. Suitable substrates include metal surfaces such as gold, electrodes as defined below, glass and modified or functionalized glass, fiberglass, ceramics, mica, plastic (including acrylics, polystyrene and copolymers of styrene and other materials, polypropylene, polyethylene, polybutylene, polyimide, polycarbonate, polyurethanes, Teflon®, and derivatives thereof, etc.), GETEK (a blend of polypropylene oxide and fiberglass), etc., polysaccharides, nylon or nitrocellulose, resins, silica or silica-based materials including silicon and modified silicon, carbon, metals, inorganic glasses and a variety of other polymers, with printed circuit board (PCB) materials being particularly preferred. This substrate is referred to herein as a "bottom substrate", although as will be appreciated in the art, in some embodiments this substrate could be on "top" or the "side", relative to the ground.

[0124] The substrate is divided into a number of distinct functional areas or zones, which can be both spatially overlapping and spatially distinct, as outlined herein. As will be appreciated by those in the art, some of these zones, for example the sample preparation zone, may be optionally included or excluded depending on the assay and/or sample.

[0125] In general, as discussed above, the microfluidic platform used herein is based on the use of electrowetting techniques to form microdroplets that can be manipulated both spatially and biochemically as further described below.

[0126] Electrowetting techniques are the basis of the microfluidic cartridges herein. Electrowetting is the modification of the wetting properties of a hydrophobic surface (such as PCB) with an applied electric field. In an electrowetting system, the change in the substrate-electrolyte contact angle due to an applied potential difference results in the ability to move the electrolyte on the surface. Essentially, as described in U.S. Pat. No. 6,565,727 in the Summary of the Invention (hereby expressly and specifically incorporated by reference), by applying an electric potential to an electrode (or group of electrodes) adjacent to a drop of polar liquid (e.g. one containing a target analyte), the surface on these electrodes becomes more hydrophilic and the drop is pulled by the surface tension gradient to increase the area overlap with the charged electrodes. This causes the drop to spread on the surface, and by subsequently removing the potential, or activating different electrodes, the substrate returns to a hydrophobic state, resulting in the drop moving to a new hydrophilic area on the substrate. In this way, the drops can be physically and discretely moved on the planar surface of the substrate to different zones, for processing, handling and detection. The drops can be moved at varied speeds, split (e.g. a single drop can be split into two or more drops), pulsed and/or mixed (two or more drops merged onto the same location and then either split or moved as one). In addition, electrowetting can instigate mixing within a single droplet. As described in more detail below, drops can also be used to rehydrate dry reagents stored at different locations on the PCB substrate. A key advantage of electrowetting is precise manipulation of very small volumes. For example, isolated target nucleic acid can be eluted at a very high concentration in less than 10 μl prior to PCR amplification, compared to 100 μl elution volumes and much lower target analyte concentrations featured in other systems. In addition, electrowetting allows altering fluid paths in development and in the product via software, without the need to make any changes to the physical interface (e.g., new valves, fluid paths, etc.).

[0127] Microfluidic systems utilizing these techniques have been pioneered by Advanced Liquid Logic, and are described in U.S. Patent Pub. Nos. 2013/0252262, 2013/0233712, 2013/0233425, 2013/0230875, 2013/0225452, 2013/0225450, 2013/0217113, 2013/0217103, 2013/0203606, 2013/0178968, 2013/0178374, 2013/0164742, 2013/0146461, 2013/0130936, 2013/0118901, 2013/0059366, 2013/0018611, 2013/0017544, 2012/0261264, 2012/0165238, 2012/0132528, 2012/0044299, 2012/0018306, 2011/0311980, 2011/0303542, 2011/0209998, 2011/0203930, 2011/0186433, 2011/0180571, 2011/0114490, 2011/0104816, 2011/0104747, 2011/0104725, 2011/0097763, 2011/0091989, 2011/0086377, 2011/0076692, 2010/0323405, 2010/0307917, 2010/0291578, 2010/0282608, 2010/0279374, 2010/0270156, 2010/0236929, 2010/0236928, 2010/0206094, 2010/0194408, 2010/0190263, 2010/0130369, 2010/0120130, 2010/0116640, 2010/0087012, 2010/0068764, 2010/0048410, 2010/0032293, 2010/0025250, 2009/0304944, 2009/0263834, 2009/0155902, 2008/0274513, 2008/0230386, 2007/0275415, 2007/0242105, 2007/0241068, U.S. Pat. Nos. 8,541,176, 8,492,168, 8,481,125, 8,470,606, 8,460,528, 8,454,905, 8,440,392, 8,426,213, 8,394,641, 8,389,297, 8,388,909, 8,364,315, 8,349,276, 8,317,990, 8,313,895, 8,313,698, 8,304,253, 8,268,246, 8,208,146, 8,202,686, 8,137,917, 8,093,062, 8,088,578, 8,048,628, 8,041,463, 8,007,739, 7,998,436, 7,943,030, 7,939,021, 7,919,330, 7,901,947, 7,851,184, 7,822,510, 7,816,121, 7,815,871, 7,763,471, 7,727,723, 7,439,014, 7,255,780, 6,773,566, and 6,565,727, all of which are incorporated by reference in their entirety for the Figures and Legends and accompanying description associated with electrowetting configurations, techniques and formation of electrowetting grids.

[0128] Thus, the substrates of the invention contain a grid of electrodes such that discrete processing zones are created, including pathways or routes for the drops as appropriate for the assays being run. In general, a "spot" or "location" or "pad" (sometimes referred to as an "electrowetting pad" or (EWP") is generally depicted in the present figures and those of the incorporated ALL patents as a square surrounded by electrodes, such that a drop moves along a path in discrete steps, from pad to pad, similar to game pieces on a game board. By manipulating the electronic grid, the drops can move in four directions as needed, forward (north), backward (south), left (west) and right (east), relative to a starting position.

[0129] As will be appreciated by those in the art, there are a wide number of electrode grid configurations that can be used to generate the multiplex cartridges of the present invention. Exemplary of an embodiment of particular use are FIGS. **20-21**, which depict a system a system with a three track amplification pathway and 5 detection subarrays in the detection zone. FIG. **25** depicts a similar embodiment with a two track amplification pathway. In some alternative embodiments, a four or five track amplification pathway can be employed. As noted above, each amplification track can